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Oxidative stability of extra virgin olive oil assessed by electron paramagnetic resonance, chemical composition, and multivariate analysis

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ABSTRACT

The oxidative stability of extra virgin olive oil (EVOO) is a key quality parameter influenced by its composition and antioxidant content. The determination of EVOO oxidative stability under stress conditions will help to address its nutritional properties and shelf life. This study investigates the oxidative behaviour of Tuscan EVOOs by combining Electron Paramagnetic Resonance (EPR), detailed compositional profiling and multivariate analysis. Significant variability in both radical generation and antioxidant capacity was observed among samples. Multivariate analyses (PCA, PLS), integrating chemical data, revealed that tocopherols, phenolic compounds, and fatty acid profiles, particularly MUFA/PUFA ratios, strongly influence oxidative resistance. In particular, given that oil samples have all an oleic acid content higher than 72 %, the polyphenol/tocopherol ratio is the main responsible of the oxidative stability of EVOOs. This study highlights the interplay between composition and oxidative stability with the aim, once a greater number of dataset will be available, to build a predictive model to define EVOOs oxidative stability.

1. Introduction

Extra virgin olive oil (EVOO) is a traditional Mediterranean product, valued for its health benefits and sensory properties. It is primarily composed of triacylglycerols, with oleic acid (a monounsaturated fatty acid - MUFA) as the main component, alongside other fatty acids (FA) such as linoleic, palmitic, and stearic acids (Revelou et al., 2021). The unsaponifiable fraction includes secondary metabolites, bioactive compounds such as tocopherols (mainly α-tocopherol), phenolic compounds divided in phenolic alcohols (hydroxytyrosol, tyrosol) and secoiridoids (e.g., oleuropein and ligstroside derivatives). These represent the majority of its polar phenols. Further compounds found in the unsaponifiable fraction are sterols (notably β-sitosterol), squalene, and pigments (chlorophylls and carotenoids). All of these minor components are largely responsible for the antioxidant, sensory, and health-promoting properties of EVOO (Jimenez-Lopez et al., 2020; Capriotti et al., 2021). During storage, EVOO undergoes chemical and physical changes that deteriorate its quality, flavour, aroma, colour, and nutritional value. Shelf-life is defined as the period after food production and packaging during which the product remains acceptable under specific storage conditions (Ün and Ok, 2018). Degradation occurs through autoxidation (in the absence of light) and photo-oxidation (under light exposure), both influenced by temperature, time, oxygen, and moisture levels. These processes result in the loss of fatty acids, tocopherols, and chlorophylls, along with the formation of oxidation products (Venturini et al., 2024). The oxidation of lipids is a key concern in edible oils, as it causes substantial alterations in their chemical composition, sensory characteristics, and nutritional value (Liu et al., 2024). Initially, oxidation occurs slowly, but it accelerates rapidly after a specific stage known as the induction period (IP) (Porcu et al., 2022). The IP reflects the overall composition of the oils, accounting for both antioxidants (polyphenols) and pro-oxidants (free fatty acids). Olive oil oxidation involves the formation of various radicals, including alkyl (R•), alkylperoxyl (ROO•), and alkoxyl radicals (RO•), which lead to the creation of hydroperoxides (ROOH) (Velasco and Dobarganes, 2002; Choe and Min,

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Abbreviations:

EVOO	Extra Virgin Olive Oil
EPR	Electron Paramagnetic Resonance
GOR	Galvinoxyl Radical
DPPH	2,2-diphenyl-1-picrylhydrazyl
PCA	Principal Component Analysis
PLS	Partial Least Squares
GC-FID	Gas Chromatograph - Flame Ionization Detector
HPLC-MS	High Performance Liquid Chromatography – Mass Spectrometry
FT-NIR	Fourier Transform Near Infrared Spectroscopy
MUFA	Monounsaturated fatty acid
FA	Fatty Acid
SFA	Saturated fatty Acid
IP	induction period
IOC	International Olive Council
PV	Peroxide value
FTIR	Fourier Transform Infrared Spectroscopy
PBN	N-tert-butyl-α-phenylnitrone
CW	Continuous Wave
T_{150}	EPR Intensity of the oil PBN-adduct signal after 150 min of incubation at T = 343 K
AUC	Area Under the Curve
Spin240	Number of spins of PBN-adducts after 240 min of incubation at T = 343 K
ID_r:	double integral of the radical probes (GOR or DPPH)
ID_X:	double integral of the radical probes (GOR or DPPH) in presence of the olive oil samples
ESI	Electrospray ionization
PCs	Principal Components
LOQ	Limit of quantification
LOD	Limit of detection
a_N	Hyperfine coupling constant for a nitrogen nucleus
a_H	Hyperfine coupling constant for a proton
EC50	Half-maximal effective concentration
FAME	Fatty Acid Methyl Ester
np:	peroxide number
PUFA	Polyunsaturate Fatty Acid
TP	Total Polyphenols
TC	Total Tocopherols
API	Apigenin
CAF	Caffeic acid
CIN	Cinnamic acid
COUM	Coumaric acid
OLT	Oleocanthal
OLA	Olacein
FER	Ferulic acid
OH-Tyr	Hydroxytyrosol
LIG	Ligstroside
LUTE	Luteolin
OLE	Oleuropein aglycone
TYR	Tyrosol
VAC	Vanillic acid
VAN	Vanillin

2006). Studying oxidative processes in EVOO is essential for understanding the mechanisms of oil degradation, optimizing the production and storage strategies, and ensuring a final product that is safe, healthy, and flavorful for consumer (Li and Wang, 2018). In this context, the development of accurate methods for monitoring oil quality is very useful. A variety of analytical techniques recognized by International Olive Council (IOC) are currently employed to evaluate the oil oxidation. Classical methods such as peroxide value (PV) are widely used to quantify primary oxidation products (Longobardi et al., 2021; Zhang et al., 2021). UV–Visible (UV–Vis) spectrophotometric indices (K232 and K270) allow rapid detection of conjugated dienes and trienes, indicating primary and secondary oxidation (Venturini et al., 2024; Malvis et al., 2019). The Rancimat test is commonly used to assess oxidative stability under accelerated conditions by measuring the induction time of volatile degradation products (García-Moreno et al., 2013). Studies showed a strong correlation between Electron Paramagnetic Resonance (EPR) derived induction periods and those from the Rancimat method, confirming EPR as a reliable and efficient tool for evaluating oil stability and shelf life (Papadimitriou et al., 2006). The EPR technique provides rapid, real-time insights into radical species generated during oxidation (Costa et al., 2025; Augusto et al., 2023; Jiang et al., 2020). In EPR, the use of spin-trapping agents stabilizes short-lived radicals, enabling the monitoring of oxidative events and antioxidant activity by tracking radical quenching (Xie et al., 2019; Fadda et al., 2023; Ottaviani et al., 2001). In this study, thirteen EVOOs derived from different cultivars grown in the provinces of Siena and Grosseto (Tuscany, central Italy) were analysed combining EPR-spin trapping data and antioxidant activity measurements with detailed chemical composition and multivariate analysis. Therefore, the combination of these datasets leads to an accurate analysis and correlation of oil components addressing oils' oxidative stability. Variations in phenolic content or fatty acid unsaturation levels can explain differences in the types and quantities of radicals detected by EPR and the efficiency of radical scavenging. In this paper's case study, the polyphenol/tocopherol ratio was identified as the primary determinant of the oil's oxidative stability, provided that the oleic acid content was higher than 72 %. These preliminary results can serve to create a predictive model to asses the oil quality on the basis of their oxidative potential.

2. Material and methods

2.1. Chemicals

The chemicals used in this study, including (N-tert-butyl-α-phenylnitrone (PBN, CAS 3376-24-7), 2,6-Di-tert-butyl-α-(3,5-di-tert-butyl-4-oxo-2,5-cyclohexadiene-1-ylidene)-p-tolyloxy (Galvinoxyl radical, GOR, CAS 2370-18-5) and 2,2-diphenyl-1-picrylhydrazyl (DPPH, CAS, 1898-66-4) and the solvents Ethanol (≥99.5 %, CAS 64-18-6), Chloroform (≥99.8 %, CAS 67-66-3), Methanol (MS grade, CAS 5373-11-5) and formic acid (99 %, CAS 64-18-6) were purchased from Sigma-Aldrich. Phenolic compounds (gallic acid CAS 149-91-7, hydroxytyrosol CAS 10597-60-1, tyrosol CAS 501-94-0, verbascoside CAS 61276-17-3, oleuropein CAS 32619-42-4, oleocanthal CAS 289030-99-5, caffeic acid CAS 289030-99-5, ferulic acid CAS 537-98-4, coumaric acid CAS 501-98-4, chlorogenic acid CAS 327-97-9, rutin CAS 250249-75-3, luteolin 7-O-glucoside CAS 5373-11-5, luteolin CAS 491-70-3 and apigenin CAS 5373-11-5) were purchased from Merck (Darmstadt, Germany). All reagents were of analytical grade and distilled water was obtained from a Milli-Q purification system from Millipore (Milford, MA, USA).

2.2. Olive oil samples

Thirteen olive oils from eight olive groves in different areas of the southwest of Tuscany (Italy, production date: November 2022) were analysed. The selected oils are listed in Table 1, which includes their codes and cultivars. These oils are either blended or monocultivar,

Table 1
Olive oil sample codes and cultivars.

n.	Oil sample codes	Cultivars
1	OL1	Blend
2	OL1_F	Frantoio
3	OL2	Blend
4	OL2_M	Moraiolo
5	OL2_P	Blend
6	OL3_M	Moraiolo
7	OL3_F	Frantoio
8	OL3_L	Leccino
9	OL4_F	Frantoio
10	OL5	Blend
11	OL6	Blend
12	OL7	Blend
13	OL8	Blend

featuring varieties such as Frantoio, Moraiolo and Leccino, all typical Italian cultivars. The olive oils were harvested and maintained at low temperature before the analysis. The analysis has been performed in triplicate for each olive oil.

2.3. EPR measurements

EPR measurements were performed in Continuous Wave (CW) X-band mode using a Bruker ELEXSYS E580 Super Q-FT spectrometer, equipped with a Bruker ER 049X microwave bridge and a ER4122 SHQE cavity. The temperature was set up by using the Bruker ER4111t variable temperature unit. Data acquisition and double integral calculation were performed using the Bruker Xepr software (2.6b.176 version). The double integral calculation of each EPR signal, expressed in arbitrary units, was used to quantify the number of spins using a procedure in the software.

The EPR spin-trapping experiments were performed using 5.0 μL of a 2.50 M PBN (N-tert-butyl-a-phenylnitrone) solution in absolute ethanol. Subsequently the solution was dried under a nitrogen flux to avoid any interference of ethanol during the experiment. Then, 100 μL of olive oil was mixed with the dried PBN, transferred into an EPR tube (3.5 × 4.0 mm) and inserted into the resonant cavity heated at 343 K. The first EPR spectrum was recorded after 20 min and subsequently every 10 min until 240 min (4 h) under continuous heating at 343 K. The spin content of the PBN-adduct was estimated from the double integration of the spectra and plotted against time (Fadda et al., 2023). The Boltzmann function shown in Supplementary material, Fig. S1, was used to fit the EPR data. The lag-time, corresponding to the Induction Period (IP), was determined as the intersection point between the slow-increase phase and the rapid-increase phase. This lag-time correlates with the onset of stale flavours and changes based on the oil's composition, making it a valuable measure for assessing the oil's antioxidant activity. Furthermore, the lag-time represents the period required to produce H_2O_2 through oxygen reduction by pro-oxidants, and it can be extended by the presence of antioxidants (Uchida and Ono, 1999). This measurement has been supplemented by additional metrics, such as the intensity of the adduct signal after 150 min of incubation at high temperature (T_{150}), the amount of PBN-spin adducts at 240 min (spin240) and the area under the curve (AUC) (Marques et al., 2017). The experimental data were fitted with Origin Pro 2025 program (licence: GF3S5-6089-7187494).

The free radical scavenging activity was assessed using two different paramagnetic probes: Galvinoxyl radical (GOR) and 2,2-diphenyl-1-picrylhydrazyl (DPPH). Both of them can be used to evaluate the endogenous antioxidant capacity but differ in steric accessibility to radical species. Due to the complex matrix of olive oil, both methods were tested and compared. GOR assay: a total of 19 mg of olive oil was dissolved in 200 μL of chloroform and added to 200 μL of GOR solution (1.10 mM in chloroform to match the final concentration of GOR in the oil samples, 0.55 mM). The mixture was then transferred in an EPR tube of 1.0 × 1.2 mm, and then inserted into a 3.5 × 4.0 mm tube for analysis. The reaction was monitored over time by recording spectra at 5, 10, 15, 30, 60, 150, 180, and 240 min (Papadimitriou et al., 2006). DPPH assay: 100 μL of the oil sample were added to 900 μL of DPPH stock solution (1.00 mM in chloroform to match the final concentration of 0.90 mM DPPH in the oil samples). The double integrals, to calculate the spin concentration, were evaluated on the EPR spectra recorded after 15 min (Naik et al., 2014; Chen et al., 2023).

The antioxidant activity was calculated using the following equation (1):

$$Antioxidant\ activity\ (\%) = \frac{ID_r - ID_X}{ID_r} \quad (1)$$

Where ID_r represents the double integral of the radical probes (GOR or DPPH), and ID_X is the double integral in presence of the olive oil samples. The measurements were performed at room temperature (298 K) and in triplicate.

2.4. EVOO chemical composition

The chemical composition of olive oils was determined following official methods IOC-Trade Standard Applying to Olive Oils and Olive-Pomace Oils (COI/T.15/NC No 3/Rev. 15) for free fatty acid (COI/T20/Doc34/Rev12017), peroxide number (COI/T20/Doc35/Rev1/2017) and fatty acid methyl esters (COI/T20/Doc33/Rev1/2017). Phenolic compounds were extracted following IOC procedure (COI/T20/Doc29/Rev2/2022) and analysed by liquid chromatography coupled with high resolution mass spectrometry (HPLC-HRMS) as reported by Borghini et al. (2024). Briefly, samples were extracted using a methanol/water mixture (80:20) for 15 min at 23 ± 2 °C in an ultrasonic bath, centrifuged (3500 rpm, 15 min), and then the samples were filtered (0.22 μm) prior to the HPLC-HRMS analysis. Chromatographic separation was carried out on a Vanquish Flex system (Thermo Fisher Scientific, Waltham, MA, USA), using a Hypersil Gold C18 column (2.1 × 100 mm, 1.7 μm; Thermo Fisher Scientific). The column temperature was maintained at 313 K. A gradient elution was applied using water (phase A) and methanol (phase B) both acidified with 0.1 % formic acid at a flow rate of 300 μL/min. The chromatographic separation was conducted over a 30min run with the following gradient: 0 min, 5 % B; 10 min, 50 % B; 12 min, 60 % B; 25 min, 90 % B; 27 min, 90 %; 28 min 5 % B followed by a 2 min equilibration step at 5 % B from 28 to 30 min. An Exploris 120 Orbitrap instrument (Thermo Fisher Scientific) with an electrospray ionization (ESI) source working in negative ion mode was used to mass detection. Full MS and data-dependent MS^2 experiment were performed with scan ranges of 90–900 m/z, at resolutions of 120,000 and 15,000 FWHM, respectively. Quantification was carried out using TraceFinder 4.1 software with external calibration curves and compounds lacking commercial standards were quantified based on structurally related analogs.

Monounsaturated fatty acids, Omega-3 fatty acids, Omega-6 fatty acids, polyunsaturated fatty acids, saturated fatty acids, total polyphenols and total tocopherols were stated by Fourier Transform Near Infrared Spectroscopy (FT-NIR) analyser (Antaris II, Thermo Fischer Scientific) in combination with multivariate calibration methodologies. Briefly, 10 mL of sample were filtered by filter paper (MN 616 ¼, 185 mm diameter) in order to remove water and particulate matter, then FT-NIR spectra were acquired in absorbance mode in the entire region of 833–2630 nm. All the analysis were performed in triplicate.

2.5. Statistical analysis

XLStat for Windows (version 2014.5.03) was used for the statistical data analysis: Spearman correlation test was conducted to investigate relation between analysed parameters, setting the level of significance at $p < 0.01$. Unscramble X was used to perform both Principal Component

Analysis (PCA) and Partial Least Square (PLS) regression. PCA is an unsupervised method of linear dimensionality reduction, which is used extensively as a way to visualize data structures and latent patterns. The goal of PCA is to identify and summarize the main patterns in the data by transforming it into a lower-dimensional space defined by principal components (PCs) that capture most of the original variance. PLS regression, on the other hand, is a supervised method used to model the relationship between predictor variables (X) and response variables (Y). Both variables are projected onto a new space of latent variables that maximize the covariance between them, making it particularly useful for predictive modeling and interpretation of complex, multicollinear datasets. PLS was performed using chemical parameters as predictors and EPR variables (spin240 and lag-time) as response variables in order to better understand the relationship between oxidative stability and chemical composition. The data matrix was normalized before the multivariate statistical analysis and for analytes with concentrations below the limit of quantification (LOQ), missing data were imputed using one-third of the corresponding limit of detection (LOD/3) values.

3. Results and discussion

3.1. Evaluation of EVOO oxidative stability by EPR

The EPR spectrum of the PBN-adduct with the six hyperfine lines and the hyperfine coupling constants is reported in Fig. 1 on the left. For all the analysed oils, the coupling constant (a_N) was 1.48 ± 0.10 mT with the presence of the nitroxyl group and a second hyperfine interaction with a hydrogen atom having a coupling constant (a_H) of 0.22 ± 0.01 mT and g = 2.0063 ± 0.0007. The line broadening and the decrease in the intensity, observed mainly for the high field lines, can be ascribed to the decreased mobility of the PBN-adduct due to the viscous nature of the oils. This combination of hyperfine coupling constants and g-value suggests the presence of PBN-lipid adducts, indicating a C-centered radical (Merkx et al., 2021) (see Fig. 1).

Fig. 1 on the right shows the evolution of the PBN-adduct as a function of time during the constant heating at 343 K. The intensity increase in the EPR signal of the adduct, shows that more radicals are formed and the antioxidant activities of the sample is reduced. After 240 min the concentration of PBN-adducts expressed in number of spins was quantified and reported in Table 2. Two monocultivar OL2_P and OL3_F reported higher concentration of adducts with approximately the same number of spins 4.96 and 4.98 x 10^{15} respectively, indicating poor oxidative stability. This analysis allows the assessment of oil oxidative stability by monitoring radical formation over time under induced oxidative stress.

The curves of the PBN-adduct at 343 K (Fig. 2) were used to extrapolate the kinetic parameters, calculated by fitting the Boltzmann function, as shown in Fig. S1 (Barr et al., 2001; Fadda et al., 2021). The curve resembles the typical kinetic profiles of peroxide value (PV)

Table 2
Fitting parameters calculated using the Boltzmann equation for PBN-adducts at 343 K over time.

Samples	Spin240 (x10^{15})[a]	Lag-time (min)	T_{150}[a]	AUC[a]
OL1	1.10 ± 0.02	40 ± 3.2	36 ± 4.0	559.4
OL1_F	1.91 ± 0.01	31 ± 1.6	49 ± 3.2	518.7
OL2	1.40 ± 0.01	26 ± 0.5	34 ± 2.0	591.8
OL2_M	2.76 ± 0.05	25 ± 3.3	69 ± 5.0	381.0
OL2_P	4.96 ± 0.06	22 ± 1.4	157 ± 6.2	672.7
OL3_M	2.22 ± 0.02	19 ± 2.5	58 ± 2.7	344.7
OL3_F	4.98 ± 0.05	20 ± 1.3	152 ± 3.7	378.1
OL3_L	1.99 ± 0.01	25 ± 2.5	51 ± 7.1	413.1
OL4_F	1.75 ± 0.02	30 ± 3.0	44 ± 3.2	484.3
OL5	1.86 ± 0.02	31 ± 2.3	48 ± 2.0	356.8
OL6	1.45 ± 0.01	42 ± 3.3	9 ± 0.5	167.9
OL7	1.89 ± 0.03	25 ± 2.0	43 ± 1.0	469.8
OL8	2.59 ± 0.05	24 ± 3.6	23 ± 2.2	331.9

[a] These values are referred to the amount of spin PBN-lipid adducts after 240 min at 343 K.T_{150} and AUC are expressed as arbitrary units. The AUC is referred to the interval 0–150 min under the curve of Boltzmann fitting.

changes observed during the initiation, propagation, and termination phases of lipid peroxidation in olive oils (Farhoosh, 2025). The fitting parameters are reported in Table S1 (Supplementary material). The R-Square value of statistical analysis was around 0.99, which means the data have high correlation. The curve fitting parameters include various values, such as EC50 (half-maximal effective concentration), which is inversely related to oil stability (Suriyatem et al., 2017).

In Table 2, the lag-time is reported. Lag-time is the most reliable indicator of food stability, longer values meaning greater oxidative resistance over time. The AUC parameter represents the evolution of PBN-adduct intensity over the first 150 min of thermal treatment, while T_{150} reflects the intensity of the spectra at 150 min. The integrated analysis of all parameters provides a reliable measure of the oil's capacity to respond radical formation under thermal stress at 343 K.

The EPR spin-trapping results revealed significant differences in radical generation and oxidative stability among the oil samples. Samples like OL2_P and OL3_F showed high radical concentrations but also high T_{150} values, suggesting poor long-term stability, indicating limited antioxidant persistence. Conversely, samples like OL6 displayed low radical presence and a long lag-time, suggesting the presence of antioxidants capable of limiting the propagation phase despite early radical formation. These results highlight the importance of integrating spin quantification with kinetic parameters to better characterize oxidative behaviour across different olive oils.

The effect of temperature variations on PBN-adduct formation was also tested and it is shown in Fig. S2 (Supplementary material). At temperatures of 343 K and 363 K, spectra exhibited similar characteristics and trends, only intensity differences were observed, with higher temperatures accelerating free radical production (Jiang et al., 2020).

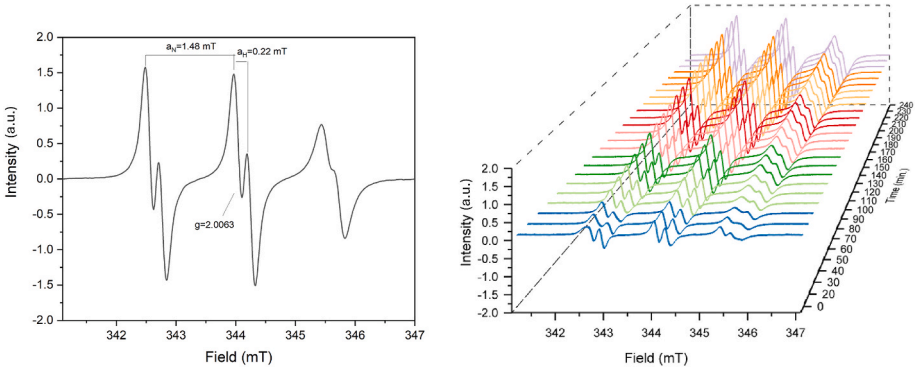

Fig. 1. EPR signal of the PBN-lipid adduct after 240 min at 343 K (left side). EPR spectra of PBN-adduct at 343 K are shown over time, from 20 to 240 min, with measurements taken every 10 min (right side). The signals correspond to OL1 sample.

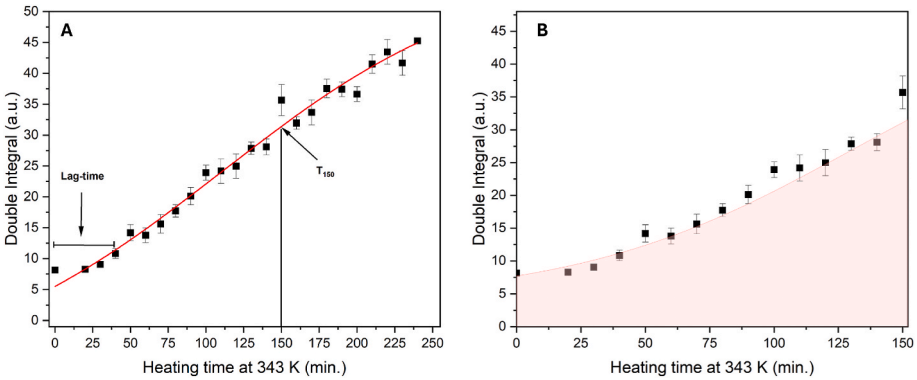

Fig. 2. Analysis of PBN-adducts formation at 343 K. A) Determination of lag-time and T_{150} using Boltzmann equation. B) Graphical representation of the Area Under the Curve (AUC) from the Boltzmann fit (0–150 min), highlighted in light red to indicate the extent of PBN-spin adduct formation. The Figures correspond to OL1 sample.

Oxidative stability depends on various factors, and the presence of antioxidants slow down the propagation phase of lipid peroxidation, thereby reducing the formation of PBN-radical adducts. The endogenous antioxidant activity in oil samples was studied using GOR and DPPH. The latter are two stable free radicals commonly employed in the evaluation of antioxidant activity. Their electronic properties, steric hindrance, and radical structure affect their reactivity profiles, offering complementary insights into the antioxidant composition of complex food matrices such as extra virgin olive oil (Koprivnjak et al., 2008; McPhail et al., 2003; Jerzykiewicz et al., 2009). Galvinoxyl, an oxygen-centered radical, is particularly sensitive to lipophilic antioxidants like tocopherols, while DPPH, a nitrogen-centered radical, is more reactive toward phenolic compounds (Brand-Williams et al., 1995).

GOR has a well-defined EPR spectrum as reported in Fig. 3A on the left with a proton coupling constant of 0.63 ± 0.01 mT and g = 2.0052 ± 0.0005 (Valcheva-Kuzmanova et al., 2012). The double integral of EPR signal was calculated after the addition of oil samples and monitored over time until 240 min. After that, the antioxidant activity was calculated for each measurement using equation (1) reported in material and methods (Section 2.3). In Fig. 3A (right), the results of the GOR assay over time are shown. The reaction stabilizes within the first 50 min, indicating that the antioxidant activity occurs rapidly and is most effective in the early stages of the reaction (Rossi et al., 2017). All of the investigated oil samples exhibited different antioxidant activity towards GOR. The Moraiolo cultivar shows the best results (dark green – OL2_M sample, and yellow – OL3_M, as seen in Fig. 3A).

The EPR spectrum of DPPH radical (Fig. 3B, on the left) presents five peaks due to the electron coupling with two equivalent nitrogen atoms

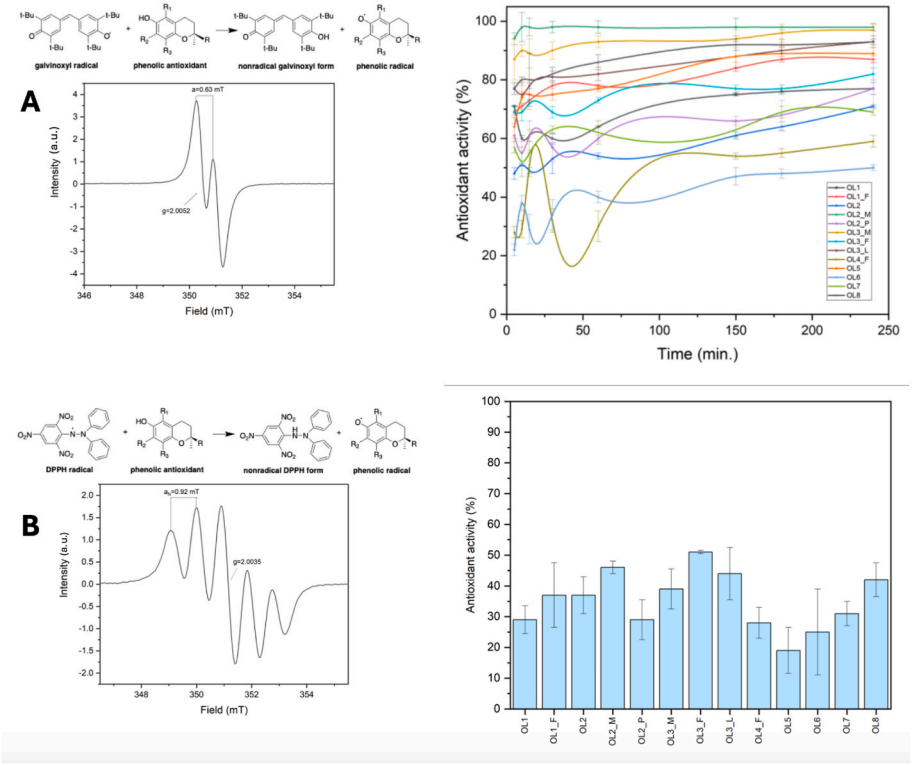

Fig. 3. Antioxidant activity analysis. The A panel shows the GOR assay, on the left, the EPR spectrum of the stable GOR radical and its reaction scheme are presented; on the right, the time-dependent antioxidant activity results for each olive oil sample. The B panel illustrates the DPPH assay, on the left, the EPR spectrum of the DPPH radical and its corresponding reaction mechanism are displayed; On the right, the antioxidant activity of each olive oil sample is shown at a fixed time point of 15 min. All the experiments were performed at room temperature and in triplicate.

with a coupling constant of 0.92 ± 0.10 mT and g = 2.0035 ± 0.0005. DPPH is a stable free radical capable to accept electrons from reactive radicals thus behaving as a radical scavenger, furthermore DPPH acts as an electron-proton acceptor from antioxidant (Gulcin and Alwasel, 2023; Zhao et al., 2018; Sheng et al., 2023). To analyse the antioxidant activity of the olive oils, the double integrated EPR signal was calculated to determine the spin content(Polovka et al., 2003). In Fig. 3B on the right, the antioxidant activity of each olive oil sample is shown at a fixed time point of 15 min. OL2_M and OL3_F exhibited the best results, along with a stable and reproducible trend. Comparing the antioxidant results obtained using the two different stable radicals reveals some differences, which can be attributed to the distinct reaction mechanisms involved. Together, these complementary approaches provide a more complete and nuanced understanding of the endogenous antioxidant activity of the samples.

Differences between EPR spin-trapping and antioxidant activity assays (GOR, DPPH) arise from their distinct principles and conditions. EPR with PBN directly detects radicals formed during heating, providing real-time insight into oxidative stress and degradation dynamics. In contrast, antioxidant assays measure the capacity of oil components (e.g., phenolics, tocopherols) to quench stable radicals at room temperature. Thus, a sample may exhibit high antioxidant activity yet still generate radicals if its antioxidants degrade under stress, or vice versa. Temperature further influences results: EPR-spin trapping operates under thermal oxidative conditions, while GOR and DPPH assays are performed at room temperature. Combining both approaches offers a comprehensive evaluation of oxidative stability, integrating radical formation dynamics and radical-scavenging capacity (Falch et al., 2005).

3.2. EVOOs composition and its correlation with EPR data through statistical analysis

To gain deeper insight into the oxidative behaviour of different olive oils, EPR results were integrated with compositional data (Tables 3–5). In all olive oil samples, secoiridoid derivatives were the most abundant compounds, followed by flavonoids, phenolic alcohols and phenolic acids, a pattern consistent with previous studies (Franco et al., 2014). However, both secoiridoids and flavonoids measured in the present study were higher than those reported for Greek (Kritikou et al., 2021) or Spanish (Franco et al., 2014) EVOOs but comparable to Italian samples (Różańska et al., 2020). Phenolic compounds can be influenced by many parameters, for instance, olive variety (Quintero-Flórez et al., 2018), agronomic factors (Caruso et al., 2017), cultivation practices (Caponio et al., 2001), as well as olive oil processing such as malaxation conditions and type of extraction system (Cecchi et al., 2018).

The FAMEs profile of the Tuscany samples was relatively homogeneous, with oleic acid levels well within the legally defined range for extra virgin olive oil (55 %–83 % of total fatty acids, by weight, IOC and the European Union Regulation Commission Implementing Regulation EU No. 2022/2104 on marketing standards for olive oil). All samples exhibited high oleic acid content, consistent with the compositional criteria established for high-quality EVOOs.

Correlation analysis between EPR-derived parameters and compositional features revealed that lag-time had a statistically significant correlation (Spearman test, $p < 0.01$) with vanillin ($r_s = 0.769$), ferulic acid ($r_s = 0.661$), acidity ($r_s = 0.752$), but also with peroxide number ($r_s = 0.598$) and total polyphenols (TP, $r_s = -0.629$, Spearman test, $p < 0.05$) indicating their possible involvement in the antioxidant capacity of the matrix. The positive ($r_s = 0.588$, Spearman test, $p < 0.05$) correlation between radical levels (spin concentration) and total polyphenols, indicates that higher phenolic content enhances the oil's resistance to oxidation due to their strong radical-scavenging capacity (Gutiérrez et al., 2001). Similarly, tocopherols, key lipid-soluble antioxidants, contribute to stabilizing radical species, although their correlation may vary depending on experimental conditions (Blekas et al., 1995; Jerzykiewicz et al., 2013). Conversely, peroxide value, which reflects primary oxidation products, showed a positive correlation with radical presence, reinforcing the relationship between oxidative progression and radical accumulation. Fatty acid composition also plays a role, higher levels of polyunsaturated fatty acids are generally associated with increased susceptibility to oxidation, while monounsaturated fatty acids, particularly oleic acid, are linked to greater stability (Fadda et al., 2023). It is important to consider that the overall scenario is complex, and discrepancies in correlations may arise from additional influencing factors, such as the presence and role of minor compounds (Gutiérrez et al., 2001; Paradiso et al., 2018). However, integrating EPR analysis with detailed compositional profiling allows for a more comprehensive evaluation of olive oil quality, offering insights into both its oxidative behaviour and protective antioxidant mechanisms. This was done by means of multivariate analysis which was used to identify specific relationships among oil samples and analytical variables, with the aim of classifying extra virgin olive oils based on their chemical composition and determining which variables served as discriminatory chemical markers of oxidative stability.

Fig. 4 reports PCA results, with both score plot and loading plot projected in PC1vs PC2 space. The first two PCs explained 43 % of the total variance (25 % and 18 % for PC1 and PC2, respectively) and divided the Tuscan olive oils into four separate groups (Fig. 4A) based on different contents of phenolic compounds. The first principal component (PC1) showed high positive loadings for MUFA, C18:1 (oleic acid), and total polyphenols (TP), and negative loadings for lag-time, peroxide value, and ferulic acid. Conversely, PC2 showed higher positive loading for luteolin, ligstroside and caffeic acid and negative ones for C16:0

Table 3
Mean free Acidity (% oleic acid), peroxide number (np, in meq O_2/kg), monounsaturated (MUFAs), omega 3-, omega 6- fatty acid (FA), polyunsaturated fatty acids (PUFAs), saturated fatty acids (SFA), total polyphenols (TP, in mg/L caffeic acid) and total tocopherols (TC, mg/kg of α-tocopherol) in EVOO oils of Tuscany. Standard deviation was <10 % for all parameters.

Sample	Acidity	np	MUFAs	Omega-3 FA	Omega-6 FA	PUFAs	SFA	TP	TC
OL1	0.52 ^{b}	12.30 ^{a}	74.90 ^{a}	7.30 ^{a}	0.73 ^{a}	8.00 ^{a}	17.0 ^{a}	369.0 ^{a}	277.0 ^{a}
OL1_F	0.32 ^{a}	11.80 ^{a}	75.50 ^{a}	6.90 ^{a}	0.69 ^{a}	7.60 ^{a}	16.8 ^{a}	578.0 ^{a}	260.0 ^{a}
OL2	0.27 ^{a}	10.30 ^{b}	76.20 ^{a}	6.50 ^{a}	0.74 ^{a}	7.20 ^{a}	16.4 ^{a}	439.0 ^{a}	245.0 ^{a}
OL2_M	0.30 ^{a}	13.50 ^{a}	75.00 ^{a}	7.00 ^{a}	0.71 ^{a}	7.70 ^{a}	17.0 ^{a}	430.0 ^{a}	226.0 ^{b}
OL2_P	0.28 ^{a}	6.70 ^{a}	75.30 ^{a}	7.10 ^{a}	0.76 ^{a}	7.90 ^{a}	16.6 ^{a}	683.0 ^{c}	235.0 ^{a}
OL3_M	0.36 ^{a}	8.60 ^{ab}	76.00 ^{a}	7.30 ^{a}	0.70 ^{a}	8.10 ^{a}	15.9 ^{a}	611.0 ^{c}	249.0 ^{a}
OL3_F	0.25 ^{c}	5.90 ^{a}	76.20 ^{a}	5.70 ^{b}	0.66 ^{a}	6.30 ^{c}	17.5 ^{a}	550.0 ^{a}	286.0 ^{c}
OL3_L	0.37 ^{a}	8.40 ^{c}	77.50 ^{a}	5.90 ^{a}	0.70 ^{a}	6.60 ^{c}	15.9 ^{a}	577.0 ^{a}	230.0 ^{a}
OL4_F	0.35 ^{a}	10.40 ^{a}	74.20 ^{b}	8.50 ^{a}	0.71 ^{a}	9.20 ^{b}	16.6 ^{a}	230.0 ^{a}	290.0 ^{c}
OL5	0.42 ^{a}	13.10 ^{a}	73.70 ^{a}	7.70 ^{a}	0.72 ^{a}	8.40 ^{a}	17.7 ^{a}	52.0 ^{a}	281.0 ^{c}
OL6	0.34 ^{a}	9.30 ^{bc}	76.70 ^{c}	5.90 ^{b}	0.72 ^{a}	6.60 ^{c}	16.5 ^{a}	194.0 ^{b}	216.0 ^{b}
OL7	0.37 ^{a}	9.00 ^{a}	74.20 ^{b}	7.10 ^{a}	0.70 ^{a}	7.80 ^{a}	17.9 ^{a}	655.0 ^{c}	251.0 ^{a}
OL8	0.52 ^{b}	12.30 ^{a}	74.90 ^{a}	7.30 ^{a}	0.73 ^{a}	8.00 ^{a}	17.0 ^{a}	369.0 ^{a}	277.0 ^{a}

Different superscript lower-case letters indicate significant differences between samples ($p < 0.05$, Kruskal-Wallis Test).

Table 4
Phenolic composition (expressed in mg/kg) of Tuscan EVOOs. ND, no detected. Standard deviation was <10 % for all parameters. API, apigenin; CAF, caffeic acid; CIN, cinnamic acid, COUM, coumaric acid; OLT, oleocanthal; OLA, olacein; FER, ferulic acid; OH-Tyr, hydroxytyrosol; LIG, ligstroside; LUTE, luteolin; OLE, oleuropein aglycone; TYR, tyrosol; VAC, vanillic acid; VAN, vanillin.

Sample	API	CAF	CIN	COUM	OLT	OLA	FER	OH-Tyr	LIG	LUTE	OLE	TYR	VAC	VAN
OL1	24.16 ^{a}	0.168 ^{ab}	0.205^{a}	0.108 ^{a}	501 ^{bc}	493 ^{a}	0.09 ^{a}	7.09 ^{a}	4.86 ^{a}	86.1 ^{a}	597 ^{a}	0.895 ^{ab}	0.042 ^{a}	0.155 ^{a}
OL1_F	36.20 ^{bc}	0.280 ^{b}	0.252 ^{a}	0.098 ^{a}	676 ^{b}	654 ^{a}	<0.01	12.46 ^{b}	7.51 ^{ab}	126.2 ^{b}	1151 ^{a}	1.141^{b}	0.054 ^{a}	0.157 ^{a}
OL2	17.08 ^{b}	0.062 ^{c}	0.111^{b}	0.045 ^{a}	183 ^{c}	255 ^{ab}	<0.01	5.13 ^{a}	2.83 ^{a}	48.3 ^{a}	436 ^{a}	0.334 ^{c}	0.039 ^{a}	0.081 ^{a}
OL2M	35.20 ^{a}	0.107 ^{a}	0.134 ^{a}	0.045 ^{a}	409 ^{a}	743 ^{a}	<0.01	4.46 ^{a}	4.55 ^{a}	35.5 ^{c}	199 ^{ab}	0.411 ^{a}	0.03 ^{ab}	0.078 ^{a}
OL2_P	25.57 ^{a}	0.151^{a}	0.153 ^{a}	0.044 ^{a}	328 ^{a}	367 ^{a}	<0.01	3.24 ^{ab}	4.71 ^{a}	86.3 ^{a}	989 ^{a}	0.564 ^{a}	0.047 ^{a}	0.098 ^{a}
OL3_M	21.43 ^{a}	0.132 ^{a}	0.340 ^{bc}	0.101 ^{a}	362 ^{a}	932 ^{bc}	<0.01	4.31 ^{a}	3.62 ^{a}	82.9 ^{a}	1297 ^{bc}	0.606 ^{a}	0.036 ^{a}	0.042 ^{a}
OL3_F	32.69 ^{a}	0.155 ^{a}	0.207^{a}	0.084 ^{a}	336 ^{a}	558 ^{a}	ND	4.67 ^{a}	11.40 ^{b}	83.3 ^{a}	1442 ^{b}	0.686 ^{a}	0.033 ^{a}	0.049 ^{a}
OL3_L	35.09 ^{a}	0.072 ^{a}	0.421^{c}	0.042 ^{a}	456 ^{a}	1152 ^{b}	<0.01	2.09 ^{c}	2.64 ^{a}	44.9 ^{a}	592 ^{a}	0.816 ^{a}	0.028 ^{b}	0.036 ^{b}
OL4_F	27.87 ^{a}	0.134 ^{a}	0.289^{a}	0.129 ^{c}	330 ^{a}	784 ^{a}	0.065 ^{a}	7.97 ^{a}	4.06 ^{a}	82.7 ^{a}	487 ^{a}	0.605 ^{a}	0.056 ^{bc}	0.209 ^{c}
OL5	38.84 ^{c}	0.106 ^{a}	0.160 ^{a}	0.055 ^{a}	340 ^{a}	391 ^{a}	0.065 ^{a}	4.64 ^{a}	3.06 ^{a}	115.6 ^{bc}	257 ^{a}	0.634 ^{a}	0.043 ^{a}	0.100 ^{a}
OL6	30.60 ^{a}	0.060 ^{c}	0.122 ^{ab}	0.098 ^{a}	232 ^{a}	253 ^{c}	0.156 ^{a}	6.19 ^{a}	2.69 ^{a}	69.2 ^{a}	178 ^{c}	0.436 ^{a}	0.067 ^{c}	0.208 ^{a}
OL7	21.34 ^{a}	0.063 ^{c}	0.123^{ab}	0.028 ^{b}	208 ^{ab}	304 ^{a}	<0.01	10.46 ^{bc}	1.90 ^{c}	65.4 ^{a}	216 ^{a}	0.387 ^{bc}	0.043 ^{a}	0.150 ^{a}
OL8	16.01^{b}	0.079 ^{a}	0.317^{a}	0.090 ^{a}	454 ^{a}	881 ^{a}	0.085 ^{a}	4.46 ^{a}	2.53 ^{a}	41.0 ^{ab}	1267 ^{a}	0.818 ^{a}	0.031 ^{a}	0.036 ^{b}

Different superscript lower-case letters indicate significant differences between samples ($p < 0.05$, Kruskal-Wallis Test).

Table 5
FAMEs (in percentage, %) in the studied samples. Standard deviation was <10 % for all parameters.

Sample	C20:0	C22:0	C20:1	C24:0	C18:2	C18:3	C17:0	C17:1	C14:0	C18:1	C16:0	C16:1	C18:0
OL1	0.31	0.09	0.27	0.04	6.7 ^{a}	0.65 ^{a}	0.04	0.09	0.01	75.1 ^{a}	13.8 ^{a}	1.2 ^{a}	1.91^{a}
OL1_F	0.32	0.09	0.26	0.04	6.8 ^{a}	0.58^{b}	0.04	0.08	0.01	75.3 ^{a}	13.6 ^{a}	1.1 ^{a}	2.02 ^{a}
OL2	0.32	0.09	0.27	0.04	6.9 ^{a}	0.67 ^{a}	0.04	0.08	0.01	74.5 ^{a}	13.9 ^{a}	1.2 ^{a}	2.06 ^{a}
OL2M	0.32	0.09	0.27	0.04	6.8 ^{a}	0.65 ^{a}	0.04	0.08	0.01	75.0 ^{a}	13.7 ^{a}	1.2 ^{a}	1.96 ^{a}
OL2_P	0.31	0.09	0.27	0.04	6.8 ^{a}	0.62 ^{a}	0.04	0.08	0.01	75.6 ^{a}	13.6 ^{a}	1.1 ^{a}	1.86 ^{a}
OL3_M	0.28	0.08	0.28	0.04	7.3 ^{a}	0.71 ^{a}	0.04	0.09	0.01	74.6 ^{a}	13.8 ^{a}	1.1 ^{a}	1.61 ^{b}
OL3_F	0.30	0.09	0.30	0.04	7.1 ^{a}	0.62 ^{a}	0.04	0.09	0.01	76.2 ^{a}	12.7 ^{b}	0.9 ^{b}	1.70 ^{b}
OL3_L	0.30	0.08	0.26	0.04	5.2 ^{b}	0.67 ^{a}	0.04	0.09	0.01	75.6 ^{a}	14.4 ^{a}	1.3 ^{a}	1.82 ^{a}
OL4_F	0.30	0.09	0.29	0.04	6.0 ^{a}	0.69 ^{a}	0.04	0.09	0.01	76.7 ^{b}	13.3 ^{a}	1.1 ^{a}	1.75 ^{a}
OL5	0.31	0.09	0.27	0.04	8.1 ^{c}	0.68 ^{a}	0.04	0.08	0.01	74.3 ^{a}	13.4 ^{a}	1.2 ^{a}	1.95 ^{a}
OL6	0.32	0.09	0.29	0.04	7.5 ^{a}	0.76 ^{ab}	0.04	0.08	0.01	72.9 ^{ab}	14.7 ^{ab}	1.5 ^{ab}	1.76 ^{a}
OL7	0.30	0.08	0.27	0.04	5.8 ^{ab}	0.59 ^{b}	0.04	0.09	0.01	76.6 ^{b}	13.2 ^{a}	1.2 ^{a}	1.85 ^{a}
OL8	0.32	0.09	0.25	0.04	7.1 ^{a}	0.69 ^{a}	0.04	0.08	0.01	73.3 ^{ab}	14.9 ^{ab}	1.4 ^{ab}	2.08 ^{ab}

Different superscript lower-case letters indicate significant differences between samples ($p < 0.05$, Kruskal-Wallis Test), no letters where no significant differences were found.

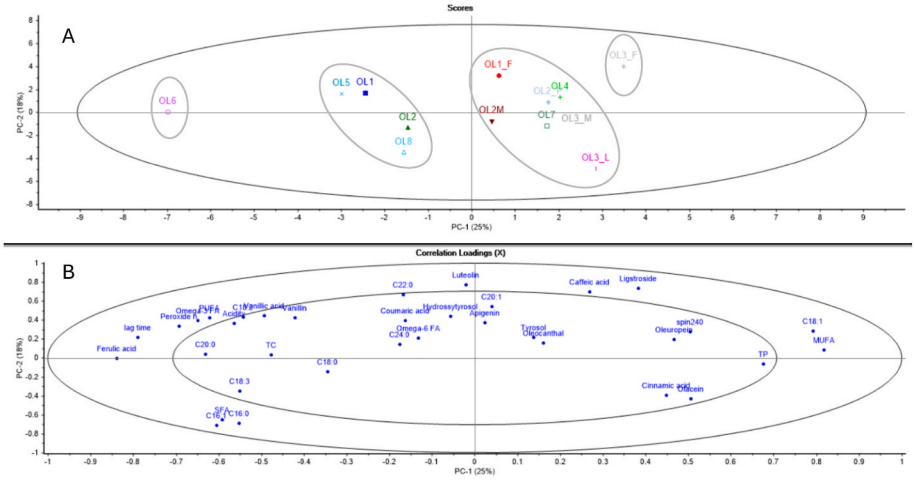

Fig. 4. Principal Component Analysis (PCA) score (A) and loading plots (B) of Tuscan olive oil samples.

(palmitic acid) and C16:1 (palmitoleic).

A multivariate PLS regression model was constructed using both EPR-derived lag-time and spin concentration at 240 s as dependent variables, despite their inverse correlation ($r = -0.821$). The approach allowed the simultaneous evaluation of predictor contributions, revealing contrasting roles for tocopherols, polyphenols and oleic acid. Loading plots (Fig. 5) showed that EPR lag-time exhibits a positive correlation with tocopherol content (TC), while showing negative correlations with both total polyphenols and oleic acid.

Although seemingly counterintuitive, these findings can be interpreted in light of the distinct antioxidant mechanisms and the physicochemical environment in which radical formation and quenching occur. The positive association between EPR lag-time and tocopherol content aligns with existing literature, as tocopherols (particularly α-tocopherol) are potent lipid-soluble radical scavengers. For instance, in stripped oils fortified with α-tocopherol, the EPR-determined lag-time significantly

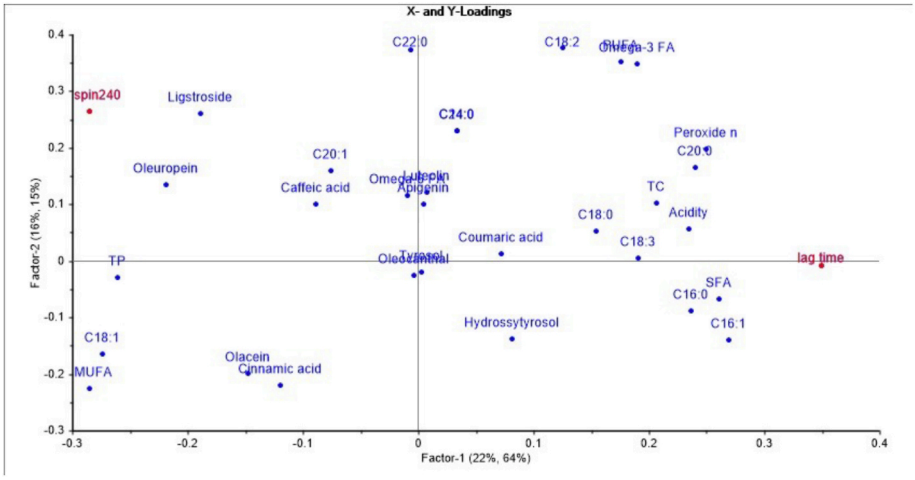

Fig. 5. Loading plots of PLS using chemical composition as predictor and EPR variables (amount of spin PBN-adducts at 240 min, indicated as spin240 and lag-time) as response variables.

increased with tocopherol dose (Cui et al., 2017). Similarly, in extra virgin olive oil samples, both lag-time and radical scavenging capacity (evaluated via PBN spin-trapping) were strongly associated with tocopherol levels (Valavanidis et al., 2004). In bulk oil systems such as those used in spin-trapping EPR, tocopherols may exert a dominant antioxidant effect by efficiently delaying the onset of radical formation, thereby extending the lag-time.

Conversely, the inverse relationship between lag-time and total phenolic content suggests that the radical scavenging capacity of polyphenols may not be linearly reflected in the EPR-derived oxidative resistance. Several factors could account for this. Polyphenolic antioxidants typically show greater efficacy in polar media or emulsified systems, in agreement with the polar paradox hypothesis, whereas they may be less effective in non-polar bulk oils (Kiokias and Oreopoulou, 2022). Moreover, the antioxidant capacity of phenolics is highly structure-dependent and total phenolic content alone fails to reflect differences in radical-scavenging efficiency among individual compounds (Moazzen et al., 2022). Hydroxytyrosol is among the most potent antioxidants found in olive oil. Its catechol structure, characterized by an ortho-dehydroxylated aromatic ring, gives the compound well-documented radical scavenging and lipid peroxidation-inhibiting (Servili et al., 2004). Similarly, oleuropein aglycone and its derivatives display strong antioxidant activity via electron transfer and hydrogen atom donation, enabled by their secoiridoid backbone and catechol moieties (Cicerale et al., 2010). Likewise, oleocanthal, a decarboxymethylated ligstroside aglycone derivative, exhibits significant antioxidant and anti-inflammatory properties, attributed to its reactive aldehyde adjacent to an unsaturated system (Jannati et al., 2025).

Conversely, tyrosol, which lacks the ortho-dihydroxyl structure, displays significantly lower antioxidant activity, despite its structural similarity to hydroxytyrosol (Servili et al., 2004). Other monophenolic compounds commonly detected in EVOO, such as vanillin and p-coumaric acid derivatives, also exhibit modest antioxidant activity, which is attributed to their limited ability to stabilize free radicals (Goulas and Manganaris, 2012). These findings highlight that both the total and qualitative phenolic composition are crucial for evaluating EVOO antioxidant potential. Under specific conditions, such as low pH or high levels of transition metals, certain phenolics may even display pro-oxidant behavior through redox cycling or metal chelation mechanisms (Chen et al., 2020).

The negative correlation between lag-time and oleic acid may reflect indirect associations rather than a causal effect. While oleic acid is known to enhance oxidative stability due to its lower degree of unsaturation compared to linoleic or linolenic acid, oils with very high oleic acid content often derive from cultivars with lower intrinsic antioxidant levels (Polari et al., 2021). As such, shorter lag-times may arise not from the fatty acid composition itself, but from lower concentrations of radical-scavenging compounds co-occurring in high-oleic samples.

Interestingly, spin240, which quantifies the cumulative radical signal after 240 min of oxidation, showed a positive correlation with oleic acid. This suggests that while oleic-rich oils may initially resist radical formation, once oxidation begins, radical species may accumulate more extensively. This could be due to lower levels of antioxidants to quench radicals formed during the propagation phase or to greater radical stability in oleic-rich matrices.

Taken together, these findings underscore the complexity of oxidation kinetics in extra virgin olive oil, highlighting the need to interpret EPR-derived parameters in the context of both antioxidant composition and fatty acid profile. The observed correlations suggest that tocopherols may be more influential than polyphenols or oleic acid in delaying radical formation.

Multivariate analysis highlights the differential mechanisms through which antioxidants and fatty acids modulate radical kinetics in EVOO. Recently, Farhoosh (2025) demonstrated that, while oleic acid contributes positively to oxidative stability due to its monounsaturated structure, its effect is not sufficient to compensate for a reduced phenolic content. Their analysis revealed that the relative contribution of the MUFA/PUFA ratio to oxidative resistance was around 20 %, while phenolic and tocopherol fractions had a more substantial role in inhibiting oxidation initiation and propagation phases, respectively.

These findings collectively support the idea that maximizing oleic acid alone does not guarantee superior oxidative stability, and that the synergistic presence of potent antioxidants, especially secoiridoids and tocopherols, is essential to achieve optimal protection against lipid peroxidation.

Considering that all oils' samples are of high quality having an oleic acid content higher than 72 %, the polyphenol/tocopherol ratio (Fig. 6) could be a valuable parameter for assessing oxidative stability given its apparent correlation with the EPR data presented in Table 2. For instance, the OL6 sample, which exhibits the greatest oxidative stability, is located within the blue region of the graph and it is characterized by a longer lag-time. In contrast, the OL3_M sample, identified as the least stable, appears in the red region of the graph and is associated with the shortest lag-time. Although additional samples are required to construct and validate a predictive model to define the shelf life of EVOO, using a combined approach of EPR data and polyphenol/tocopherol ratio, this study provides a preliminary useful framework to define EVOO oxidative stability discriminating between high quality oils with very similar origin.

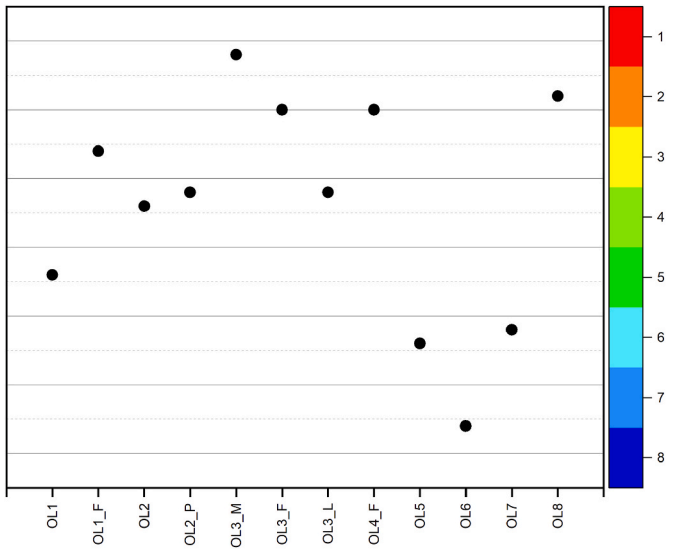

Fig. 6. Representation of EVOO oxidative stability based on the polyphenol/tocopherol ratio. The scale on the right "quality scale" indicates oil stability, with colours ranging from red (lower stability) to blue (higher stability).

4. Conclusions

This study demonstrates the value of combining EPR spin-trapping, antioxidant assays, and compositional analysis to assess EVOO oxidative stability. Fast detection of radical species with PBN under thermal stress provided key insights into lipid oxidation dynamics, revealing differences in radical kinetics and oxidative resistance among samples. Kinetic parameters from Boltzmann fitting (lag-time, spin240, T_{150}) effectively described oil stability. Integrated with antioxidant assays (GOR, DPPH), results confirmed the complementarity of the methods: EPR-spin trapping monitors real-time oxidation under stress, while antioxidant assays indicate endogenous radical-scavenging potential under milder conditions. Some oils showed strong antioxidant activity but limited oxidative stability, highlighting the importance of antioxidant persistence and oil matrix interactions. Multivariate analyses (PCA, PLS) linked stability to composition, especially tocopherols, certain phenolics (vanillin, ferulic acid), and the MUFA/PUFA ratio. Tocopherols correlated with longer lag-times, while polyphenol effects varied by structure. Overall, a multidimensional approach combining radical detection, antioxidant profiling, and compositional data enhances understanding and evaluation of EVOO oxidative behaviour. Moreover, further studies based on a larger sample dataset could allow to build a predictive model to define EVOO oxidative stability.

CRediT authorship contribution statement

Jessica Costa: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Maria Camilla Baratto: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Francesca Borghini: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization Raffaello Nardin: Writing – review & editing, Visualization, Formal analysis. Angelo Riccaboni: Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition. Rebecca Pogni: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2025.101263.

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